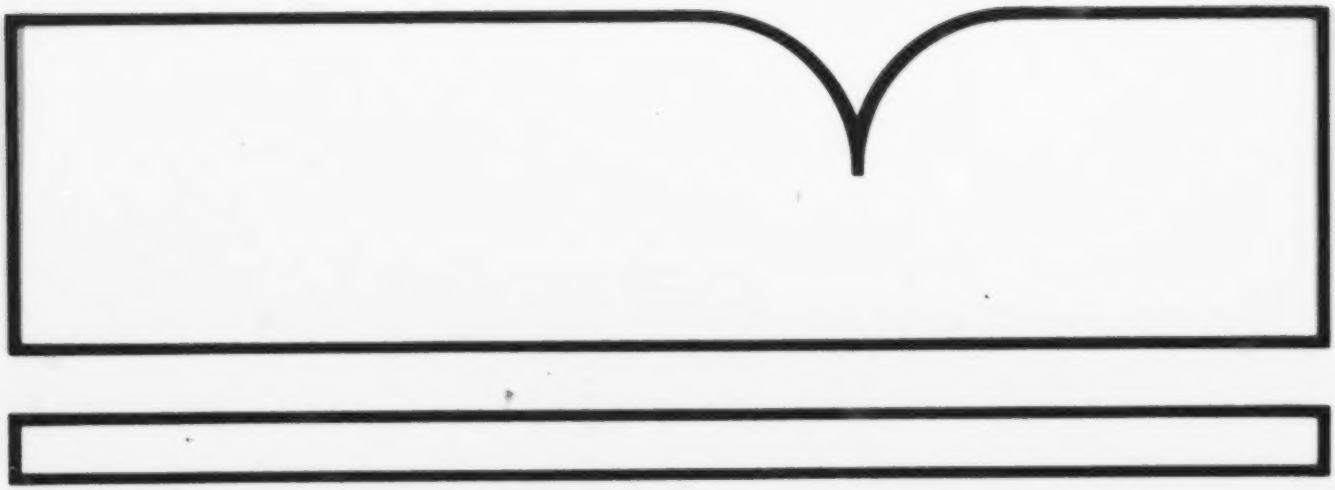
PB87-145504

Flavor Aversions Induced by Thallium Sulfate: Importance of Route of Administration

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1986



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1. REPORT NO. EPA/600/J-86/187	2.
3. PB87-145504	
4. TITLE AND SUBTITLE: Flavor aversions induced by thallium sulfate: — Importance of route of administration (Journal Version)	5. REPORT DATE 1986
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): D.B. Peele, R.C. MacPhail and J.D. Farmer	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: EPA - Neurotoxicology Division/HERL (MD-74B) RTP, NC 27711	10. IOGRAM ELEMENT NO. ACSL1A
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: Health Effects Research Laboratory Office of Research and Development US Environmental Protection Agency Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Neurobehavioral Toxicology and Teratology, Vol. 8, pp. 273-277, 1986

16. ABSTRACT

Peele,D.B., R.C.MacPhail and J.D.Farmer. Flavor aversions induced by thallium sulfate: Importance of route of administration. Neurobehavioral Toxicology and Teratology, 00: 00-00, 1985. Flavor aversions induced by intraperitoneal (i.p.) and oral (p.o.) administration of thallium sulfate were compared in a repeated trial, two-bottle preference test. Male Long-Evans rats (N=6/group) were given 30-m access to a 0.1% saccharin solution followed 20-m later by either i.p. or p.o. thallium sulfate (2.5, 5, 10 or 20 mg/kg), vehicle or nothing. Non-treated and vehicle-treated rats consistently preferred the saccharin solution, with relative saccharin intakes ranging from 0.65 to 0.85 over the three choice trials. On the first choice trial, flavor aversions produced by i.p.-administered thallium sulfate were marginal and occurred only at the highest dosage. In contrast, on the first choice trial, p.o.-administered thallium sulfate led to pronounced aversions at all but the lowest dosage. Saccharin preferences on the second and third choice trials resembled those obtained on the first choice trial. These results suggest that failure to obtain toxicant-induced flavor aversions may be due in part to the particular route by which the toxicant is administered.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT: Release to Public	19. SECURITY CLASS (This Report): Unclassified	21. NO. OF PAGES: 7
	20. SECURITY CLASS (This page): Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA/600/J-86/187
JOURNAL ARTICLE

Neurobehavioral Toxicology and Teratology, Vol. 8, pp. 273–277, 1986. Printed in the U.S.A.

Flavor Aversions Induced by Thallium Sulfate: Importance of Route of Administration[1]

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Received 6 July 1985

PEELE, D. B., R. C. MACPHAIL AND J. D. FARMER. *Flavor aversions induced by thallium sulfate: Importance of route of administration.* NEUROBEHAV TOXICOL TERATOL 8(3) 273–277, 1986.—Flavor aversions induced by intraperitoneal (IP) and oral (PO) administration of thallium sulfate were compared in a repeated trial, two-bottle preference test. Male Long-Evans rats (N=6/group) were given 30-min access to a 0.1% saccharin solution followed 20-min later by either IP or PO thallium sulfate (2.5, 5, 10 or 20 mg/kg), vehicle or nothing. Non-treated and vehicle-treated rats consistently preferred the saccharin solution, with relative saccharin intakes ranging from 0.65 to 0.85 over the three choice trials. On the first choice trial, flavor aversions produced by IP-administered thallium sulfate were marginal and occurred only at the highest dosage. In contrast, on the first choice trial, PO-administered thallium sulfate led to pronounced aversions at all but the lowest dosage. Saccharin preferences on the second and third choice trials resembled those obtained on the first choice trial. These results suggest that failure to obtain toxicant-induced flavor aversions may be due in part to the particular route by which the toxicant is administered.

Flavor-aversion conditioning Thallium sulfate Route of administration Rats

THALLIUM (Tl) is a class IIIa metal whose widespread use as a rodenticide has been responsible for a number of poisonings in human and animal species [4]. In clinical cases of thallium intoxication a syndrome of 'dying-back' neuropathy occurs along with a number of neurological symptoms such as paresthesia, hypotonia and ataxia, anorexia and visual disturbances [5, 15, 16, 23]. Despite these reported signs of neurotoxicity, there are virtually no data currently available in the literature on the behavioral assessment of thallium-treated animals.

One behavioral paradigm that has received widespread acceptance in behavioral pharmacology and increasing usage in toxicology is flavor-aversion conditioning. According to this paradigm, rats exposed to a toxic chemical after consuming a novel flavor (e.g., saccharin) will avoid its consumption on subsequent availability. A wide range of chemicals is capable of inducing flavor aversions after a single flavor-chemical pairing [27].

An experiment by Nachman and Hartley [26] determined the flavor aversions induced by intraperitoneal (IP) administration of several rodenticides, including thallium sulfate. The IP administration of thallium sulfate produced only a slight (but statistically significant) aversion at a dosage that also produced overt signs of intoxication. On the basis of these and other data, Nachman and Hartley [26] concluded that flavor-aversion conditioning was limited as an index of behavioral toxicity by its apparent lack of sensitivity to known toxicants. A number of methodological details must first be considered, however, before concluding that thallium administration is an ineffective (or weakly effective) agent for inducing flavor-aversion conditioning. For instance, in the Nachman and Hartley study, the following procedures were employed: (a) a single dosage of the compound, (b) a single route of administration, (c) a single conditioning trial, and (d) a single-bottle test. Any or all of these procedural details could conceivably lead to erroneous conclusions regarding the effectiveness of thallium administration in producing conditioned flavor aversions. Of particular interest in the present experiment was the route of thallium administration. While little data exist on the importance of route of administration in determining thallium sulfate's toxic effects, other salt forms of thallium (e.g., thallium oxide) are much more potent (i.e., lower LD50 values) when administered orally than when administered IP [9].

The present experiment was designed to determine further the conditions under which thallium sulfate induces conditioned flavor aversions. This experiment compared the effects of a range of dosages of thallium sulfate that were delivered either IP or orally (per os, PO). Flavor aversions were measured by relative (as opposed to absolute) saccha-

[1]This paper has been reviewed by the Health Effects Research Laboratory and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

[2]Supported by a National Research Council Resident Research Associateship.

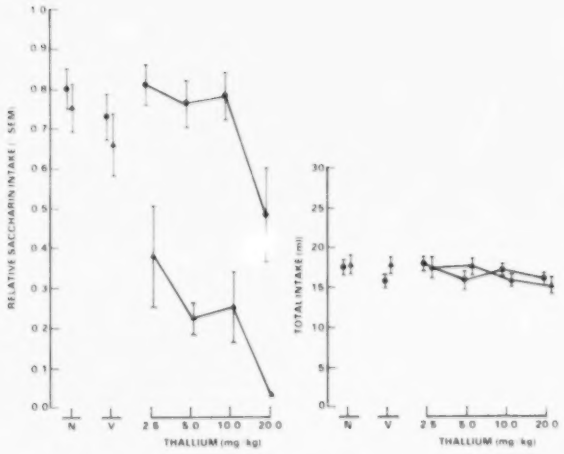

FIG. 1. Flavor aversions induced by thallium sulfate during the first choice trial. The left panel of the figure represents the average effect ±SEM of no treatment (N), the distilled water vehicle (V) or dosage of thallium sulfate on the saccharin intake of rats (n=6/group), expressed as a proportion of the total intake (relative saccharin intake). Rats received thallium (or vehicle) either IP (filled circles) or PO (filled triangles). Effects of each treatment on total intake are shown in the right panel of the figure.

rin intake in a two-bottle preference test, which has proven to be a more sensitive index of flavor-aversion conditioning (e.g., [13]). The present experiment also included multiple saccharin-thallium pairings in order to further enhance the sensitivity of flavor-aversion conditioning for detecting the effects of thallium sulfate [2].

METHOD

Subjects

Seventy-two male Long-Evans rats (Charles River, Wilmington, MA), 40 days old at the start of the experiment, served. Rats were individually housed in ceiling-suspended metal cages with unlimited access to food (Purina Rat Chow). The rats were housed in a colony room with regulated temperature (21±1°C) and relative humidity (55±5%), and a 12-hr on/12-hr off photoperiod (lights on at 0600 hr).

Apparatus

All testing was arranged in the home cages, moved to a separate testing room, between 0800 and 1000 hr. The home cages (Hoeltge Manufacturing Company, Chicago, IL), had interior dimensions of 25.4×17.8×17.8 cm with a 1.4-cm wire mesh floor and cage front. Fluids were presented in 50-ml plastic centrifuge tubes (Nalgene Co., Rochester, NY) equipped with rubber stoppers and curved stainless-steel drinking spouts. The tubes were held in place by spring clips attached to the front of the cages, with drinking spouts centered 3.8 cm above the cage floor. When two fluids were presented simultaneously, the tubes were centered on the front wall with the spouts separated by approximately 1.4 cm, and the left-right placement of the two bottles was counterbalanced.

Procedure

The rats were initially exposed to a water-deprivation schedule that allowed 30-min access to tap water each day beginning at 0800 hr. Total daily intakes were calculated from drinking tube (and spout) weights (measured to the nearest 0.1 g) determined before and after each access period. The rats received restricted water availability until daily intakes remained stable (SEM for group intakes <1 ml) for four of five consecutive days (14 days).

On Day 15, a 0.1% (w/v) sodium saccharin solution was presented in lieu of tap water for 30 min. Approximately 20-min later, 5 groups of rats (N=6/group) received IP injections of thallium sulfate (ICN Pharmaceuticals Inc., Plainview, NY) or the distilled water vehicle in dosages of 0 (vehicle alone), 2.5, 5.0, 10.0, or 20.0 mg/kg b.wt. (dosages expressed as the salt and delivered in a volume of 1 ml/kg b.wt.). The remaining group of rats served as non-injected controls. A second set of 6 groups of rats (N=6/group) was exposed to identical conditions except that thallium sulfate or the distilled water vehicle was administered PO.

On Days 16 and 17 all rats were again exposed to the limited-water-availability schedule. On Day 18, both tap water and the 0.1% saccharin solution were concurrently available for 30 min (Choice One), and were followed 20 min later by injections (IP or PO) of thallium sulfate or vehicle. This cycle of a choice trial followed by two water-recovery sessions was repeated for a total of three saccharin-thallium pairings and three choice trials.

Total intake (ml saccharin intake + ml water intake) and relative saccharin intake (ml saccharin intake/total intake) during each choice trial comprised the major data. The effect of thallium on relative saccharin intake across all choice trials was assessed with a repeated-measures analysis of variance. Due to attrition in the 20-mg/kg groups (see below), the analysis was restricted to the remaining 5 groups of IP-treated and PO-treated rats. Tukey's Studentized Range test was used for all mean contrasts [8].

RESULTS

The restricted water-availability schedule led to stable levels of water consumption within the first 14 days of the experiment; group intakes on Days 10 to 14 ranged from 18.0 to 19.3 ml (mean±SEM = 18.3±0.84 ml). Individual intakes ranged from 16 to 21 ml (range of SEMs: 0.77 to 0.99 ml).

Flavor Aversions

Relative saccharin intakes during the first choice trial for IP- and PO-dosed rats are represented in the left panel of Fig. 1. As shown in the left panel of Fig. 1, IP-vehicle and non-injected controls showed a marked preference for saccharin (relative intakes of 0.74 and 0.80, respectively) during the first choice trial. Over the range of IP-dosages tested, only rats receiving 20 mg/kg consumed less than half of their total intake from the saccharin bottle (mean relative saccharin intake=0.48). As with the IP-dosed rats, PO-vehicle and non-injected control groups showed preferences for saccharin (relative intakes of 0.66 and 0.75, respectively). Unlike the preference data observed for rats receiving thallium IP, relative saccharin intake on the first choice trial for rats receiving thallium sulfate PO was less than 0.50 at all dosages, with relative saccharin intakes ranging from 0.38 for the 2.5-mg/kg group to 0.03 for the 20-mg/kg group. As shown in

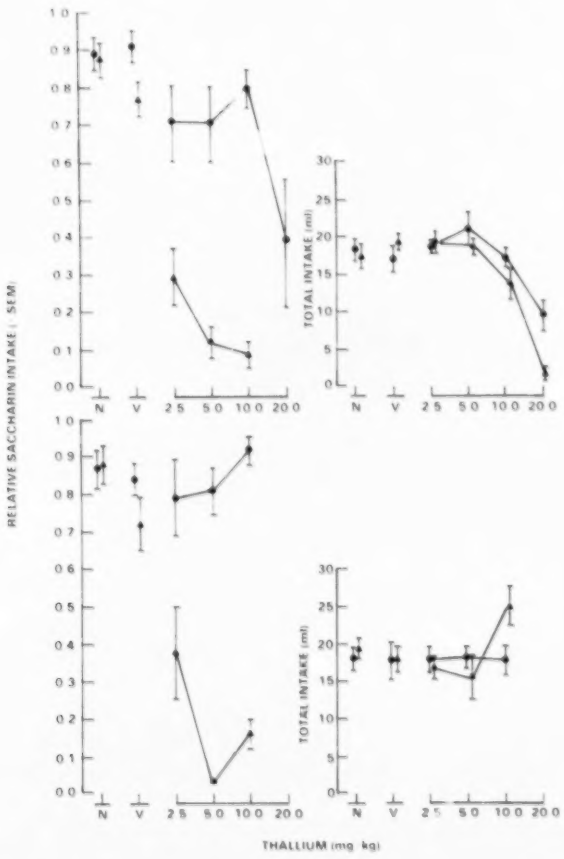

FIG. 2. Flavor aversions induced by thallium sulfate during the second (top panels) and third (bottom panels) choice trials (see legend of Fig. 1 for details). Data following 20 mg/kg were omitted due to attrition or excessively decreased total intake (see text).

the right panel of Fig. 1, total intake did not differ with respect to dosage or route of thallium administration.

Relative saccharin and total fluid intakes on choices two and three are shown in Fig. 2. Overall, the results resembled those obtained on the first choice trial. On choice trials two and three, rats receiving thallium sulfate (or vehicle, or no treatment) via the IP route of administration continued to show marked saccharin preferences, while rats receiving thallium via the PO route showed a persistent saccharin aversion at all dosages. An ANOVA showed a significant effect of dose in rats receiving thallium via the PO route of administration, $F(4,23)=31.00$, $p<0.0001$, with no significant effect of repeated pairing or dose-by-trial interaction. Conversely, the IP-treated rats showed no significant effect of dose, $F(4,25)=2.29$, n.s., repeated pairing or dose-by-trial interaction.

Total fluid intake (right panels of Fig. 2) for both IP- and PO-dosed rats remained relatively stable during choices two and three, with a couple of notable exceptions. During the second choice trial, the 20 mg/kg PO group consumed a mean of less than 3 ml, and since this value represented less than

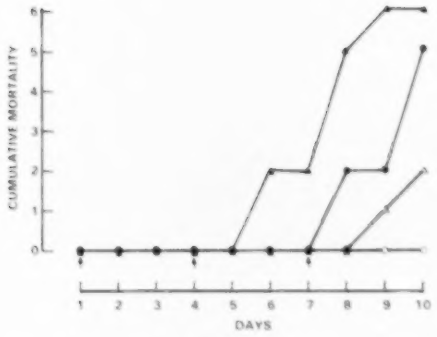

FIG. 3. Cumulative number of deaths following the IP (circles) or PO (triangles) administration of thallium sulfate. The filled symbols represent cumulative mortality of rats which received 20 mg/kg and the open symbols represent cumulative mortality of rats which received 10 mg/kg thallium sulfate. The arrows represent days on which rats received injections.

20% of baseline fluid intake, these data were excluded from the analysis of relative saccharin intake. Relative intake data on the third choice trial for both the IP and PO 20-mg/kg groups were also excluded from analysis due to attrition (see below).

Attrition

Repeated administration of thallium sulfate led to 8 deaths in PO-dosed rats and 5 deaths in IP-dosed rats, as shown in Fig. 3. Where there were enough deaths to allow comparison, the time to death was shorter following PO-administered thallium.

DISCUSSION

The data from the present experiment indicate that the temporal pairing of saccharin consumption with IP administration of thallium sulfate to rats produced a slight conditioned flavor aversion, but only at the largest dosage (20 mg/kg). Thus these results confirm and extend the findings of Nachman and Hartley [26] by showing that IP thallium sulfate does not produce robust flavor aversions in rats even when a more sensitive two-bottle test for flavor-aversion conditioning is used and a series of flavor-thallium pairings is arranged.

Unlike the data for rats receiving thallium IP, thallium sulfate administered PO after saccharin consumption induced a robust, dosage-dependent aversion to saccharin. Other compounds that have shown route-specific effects on flavor-aversion conditioning include amphetamine [6,12], trichloromethane [17], toluene [24], cadmium [22] and harmaline [3]. Still other compounds do not seem to share this route-specific effect on flavor-aversion conditioning and include delta-9 tetrahydrocannabinol [10] and lithium chloride ([1, 25, 28] but see [29] for intraventricular vs. IP lithium-chloride-induced flavor aversons). Unfortunately, most studies reporting differential effects of route of administration on flavor-aversion conditioning provide little information regarding the possible mechanisms responsible for those differences.

It is tempting to attribute the route-specific nature of the

flavor aversions induced by thallium sulfate to differing rates of absorption. As suggested by Nachman and Hartley [26], the lack of robust aversions produced by IP-administered thallium could be due to a delayed onset of effects, a delay perhaps specific to the IP route of administration. Nachman and Hartley reported that observable signs of thallium intoxication emerged some 24 hours post-dosing, thus exceeding in all likelihood the temporal boundary necessary for the formation of a conditioned flavor aversion. For example, little if any saccharin aversion is produced when delays greater than 8 hours separate consumption of saccharin and the administration of lithium chloride [11]. In the case of thallium, however, the available data on absorption and distribution fail to support this explanation. For example, Lie, Thomas and Scott [20] reported equivalent rates of absorption for between IP and PO (as well as other routes of) administration of thallium, with approximately 90% of the administered dose present after 1 day and a clearance half-time of 3.3 days for all routes of administration. When administered either IP or PO, thallium appears in the urine of rats within 30 min after dosing [20,21], with no significant difference in the mean percentage body burden of thallium as a function of route of administration [20].

An alternative explanation of these results is that the flavor aversions induced by PO-administered thallium sulfate are due to irritation of the gastrointestinal (GI) tract, an irritation not produced when the compound is administered IP. Whereas the degree of GI irritation might depend on the dosage of thallium administered, it remains to be seen whether the flavor aversions produced by PO-administered thallium are due solely to its irritant properties. Compounds reported to induce 'painful' symptoms (e.g., gallamine triethiodide) are more likely to induce associations with environmental (i.e., visual cues) stimuli than with gustatory ones, whereas the reverse is true with compounds reported to induce 'illness', such as lithium chloride [19]. Accordingly one would not expect the flavor aversions induced by orally-administered thallium to be due to pain/gastric irritation alone. A more parsimonious explanation of the present results is, perhaps, that the PO-specific flavor aversions reflect the enhanced (i.e., time to death) toxicity of thallium sulfate when administered orally. While the influence of route of administration on thallium sulfate-induced lethality has not been directly assessed, previous research on thallium oxide has indicated that route of administration might influence the toxicity of this compound. The reported LD50 value of thallium oxide delivered to rats via the IP route is 72 mg/kg, while that produced by the oral administration of the compound is 39 mg/kg [9].

While the data from the present experiment do not specify the exact mechanism(s) by which PO administration of thallium enhances its efficacy in flavor-aversion conditioning, they do suggest that a failure to produce conditioned flavor aversions might reflect a methodological inadequacy rather than a property of the agent itself. This applies equally to reported differences in the minimally effective dosages required to induce flavor aversions. For instance, Dantzer [7] reported that flavor aversions were induced by IP inorganic lead at dosages lower than 20 mg/kg. Leander and Gau [18], however, reported PO lead-induced conditioned flavor aversions at 100 mg/kg (2-bottle test) or 400 mg/kg (1-bottle test). While procedural differences between these studies make direct comparisons difficult, it appears that lead-induced flavor aversions are also influenced by the route of administration, albeit in a manner opposite to that exhibited by thallium sulfate. Future research should take into account the importance of such a procedural detail in designing flavor-aversion conditioning experiments.

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